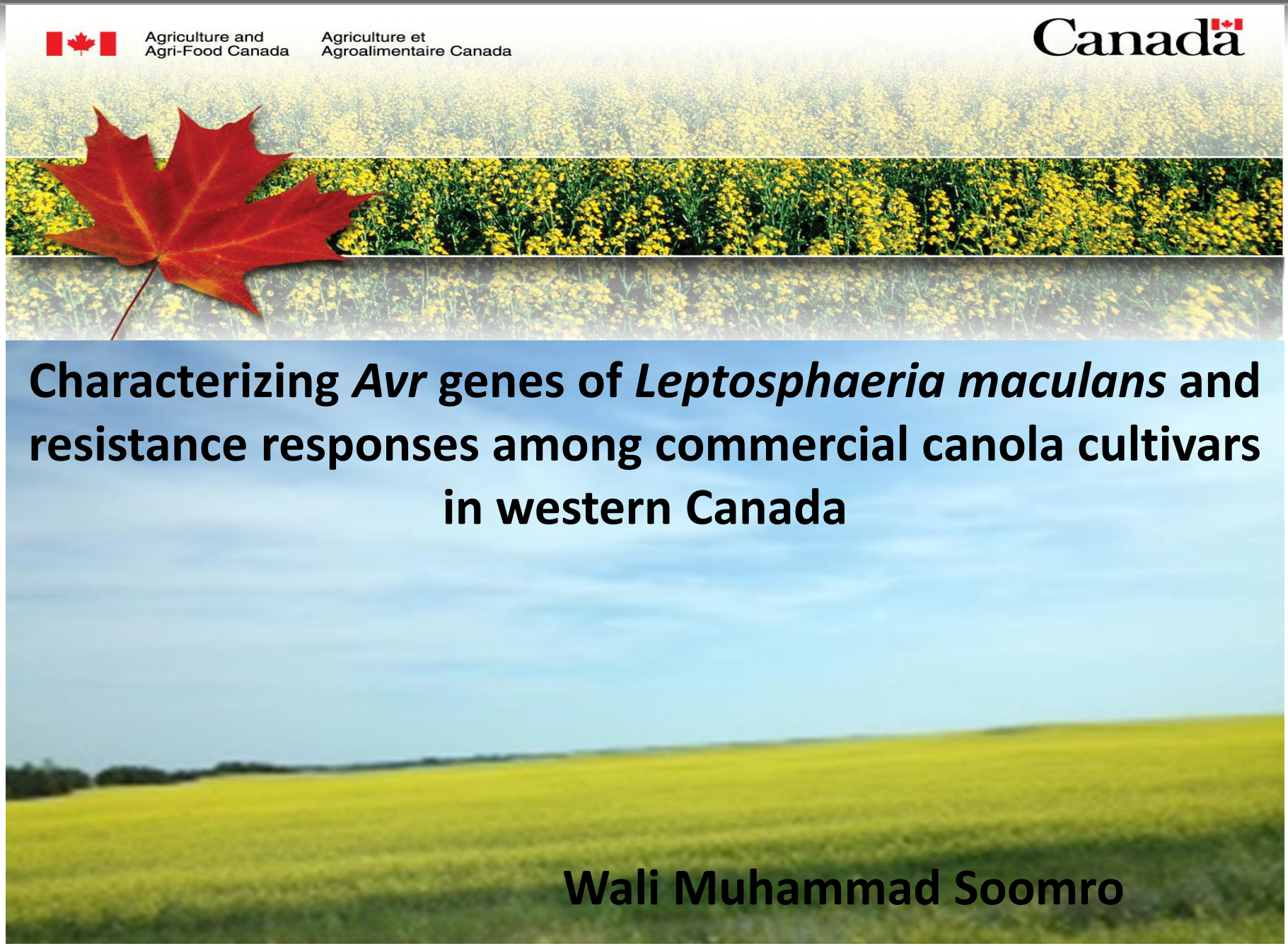
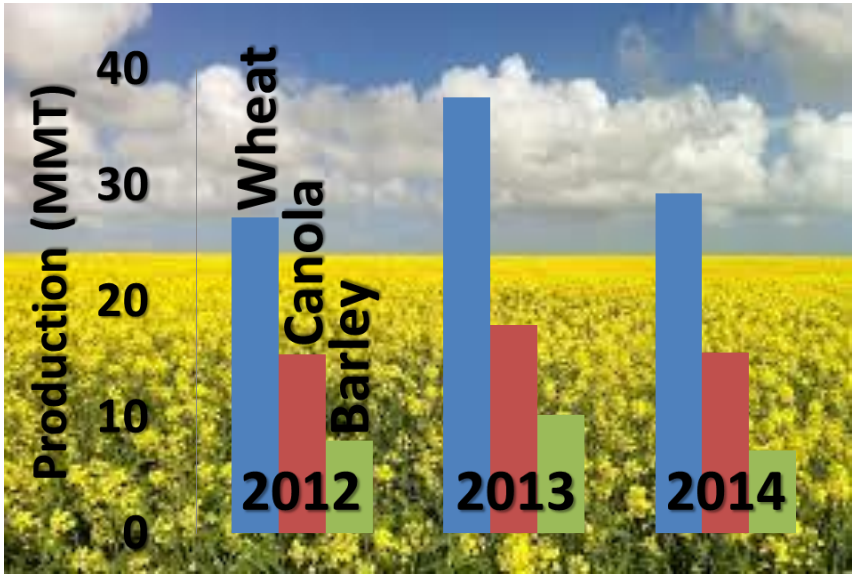


Characterizing *Avr* genes of *Leptosphaeria maculans* and resistance responses among commercial canola cultivars in western Canada

Wali Muhammad Soomro

Importance of canola



- 17.2 MMT in 2015
- > $19.3 B
- Provide jobs to 249,000
- 43,000 farms

- 90% of the canola produced is exported
- China imports canola worth $2.5 B
- China is concerned about *L. maculans* in imported canola seed

Blackleg (*L. maculans*)

- Yield and quality losses
- Spores infect cotyledons
- Fungus moves to basal stem via petiole
- Blackening inside the stem, basal canker

Plant Resistance

- Qualitative resistance
- Quantitative resistance

L. maculans displays high evolutionary potential to adopt to resistance (*R*) genes, as illustrated by breakdown of *Rlm1* in France and *LepR3* in Australia

The frequency of *Avr* genes in pathogen populations have been studied in Europe, Australia and Canada

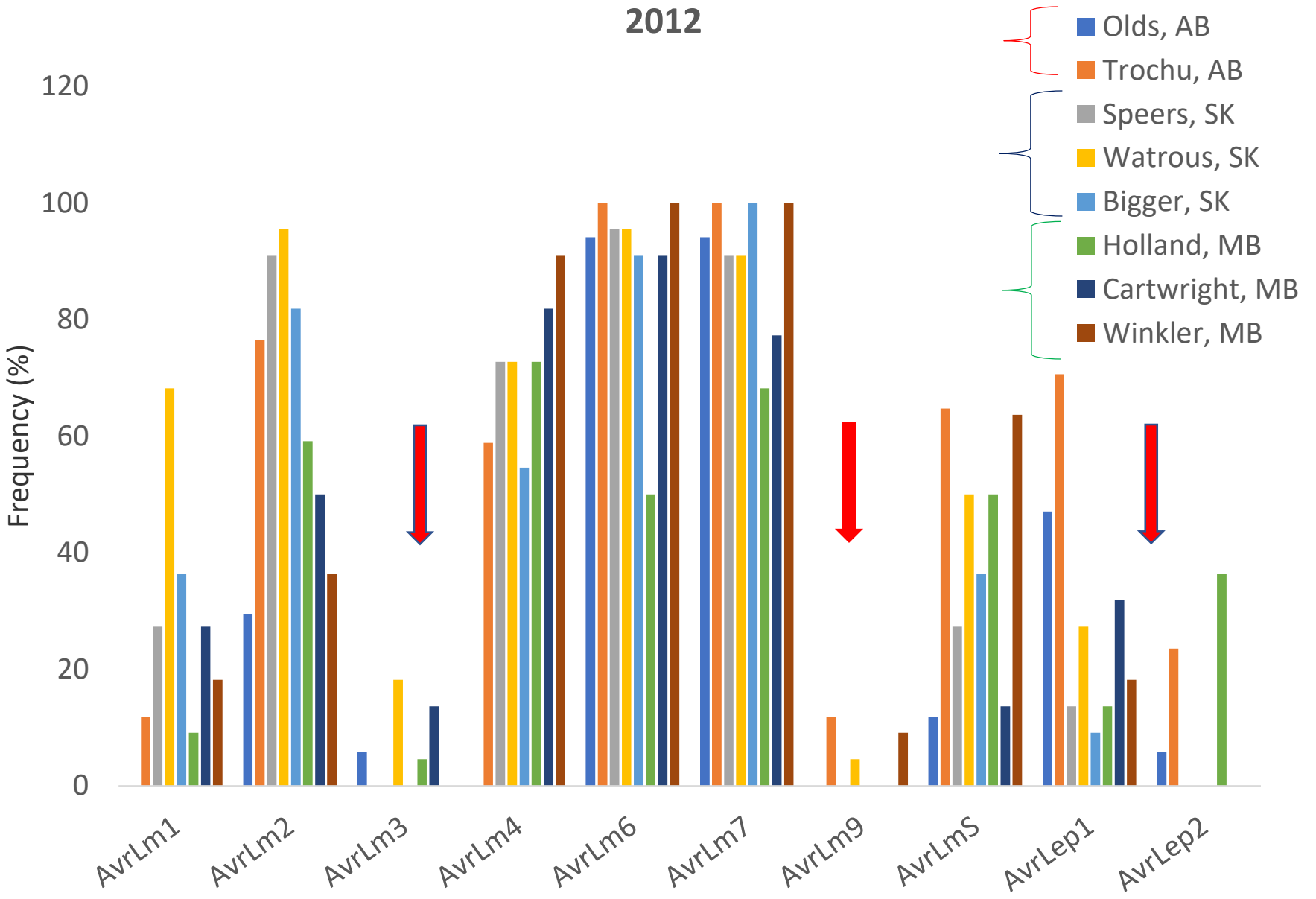
Management

Previously blackleg was managed successfully in western Canada with *R* cultivars and crop rotation, this disease is making a comeback, despite widespread use of resistant canola cultivars

Recent rise in incidence and severity of blackleg has been attributed to a shift in the race structure of *L. maculans*

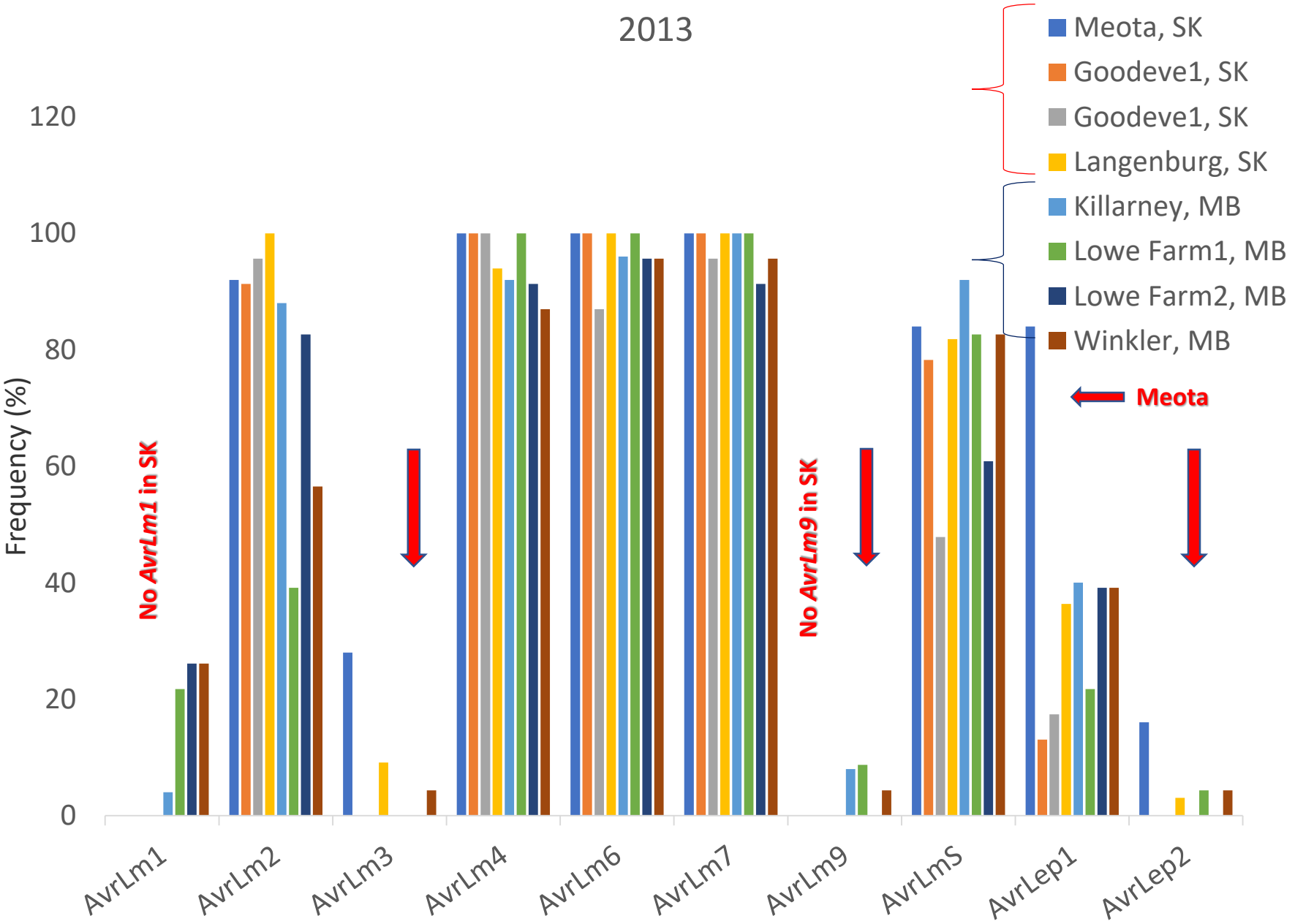
To address this issue *Avr* genes in the pathogen population were profiled in canola fields of western Canada

To do that in 2012 179 samples collected from 8 severely disease fields in AB, SK and MB were analyzed with yhe objective to determine whether the *Avr* gene profile play a role in causing different levels of diseases in these fields


2012
Olds, AB
Trochu, AB
Speers, SK
Watrous, SK
Bigger, SK
Holland, MB
Cartwright, MB
Winkler, MB
Frequency (%)
120
100
80
60
40
20
0
AvrLm1
AvrLm2
AvrLm3
AvrLm4
AvrLm6
AvrLm7
AvrLm9
AvrLmS
AvrLep1
AvrLep2

In 2013, blackleg was less sever in many regions of western Canada, specially in Alberta, as such the samples were collected from SK and MB

193 samples collected from 8 commercial fields of canola in SK and MB were analyzed to determine the frequency of *Ave* genes of *L. maculans* in these fields


2013
Meota, SK
Goodeve1, SK
Goodeve1, SK
Langenburg, SK
Killarney, MB
Lowe Farm1, MB
Lowe Farm2, MB
Winkler, MB
Meota
Frequency (%)
120
100
80
60
40
20
0
No AvrLm1 in SK
No AvrLm9 in SK
AvrLm1
AvrLm2
AvrLm3
AvrLm4
AvrLm6
AvrLm7
AvrLm9
AvrLmS
AvrLep1
AvrLep2

Summary

- Variation in *Avr*-gene frequency between fields and regions was observed
- Often the frequency of *Av1, Av3, Av9* and *AvLep2* was very low, while that of *Av6* and *Av7* was high
- Generally, the absence of *Av1, Av3, Av9* and *AvLep2* means cultivars carrying the corresponding *R* genes *Rlm1, Rlm3, Rlm9* or *LepR2* would not be effective in those fields (sites)
- ~70% of commercial canola cultivars (CCC) may carry *Rlm3* resistance gene (Zhang et al. 2015)
- *Av3* was generally low, but the disease was still isolated

Questions:

- Why is blackleg damage not more widespread?
- Are there any additional factors that play a role?
- >50% of commercial canola cultivars (CCC) may carry a level of quantitative resistance (Zhang et al. 2015)
- How does race non-specific resistance work?

Methodology

Three cultivars along with Westar as a susceptible control were selected

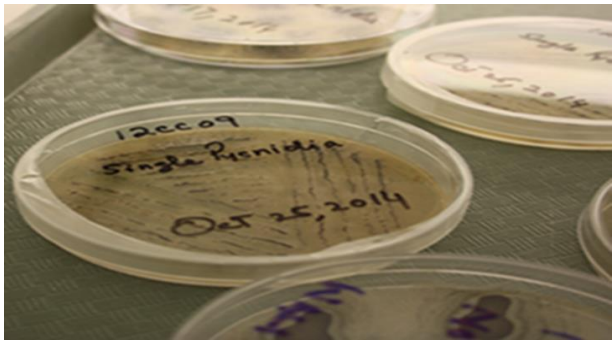
The isolates of *L. maculans* carrying no *Av1* or *Av3* (assumed to be virulent) were selected to inoculate CCCs

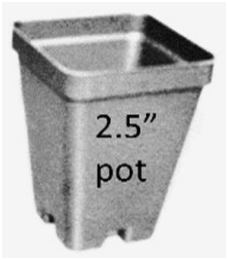
Selected CCCs were seeded in small pots

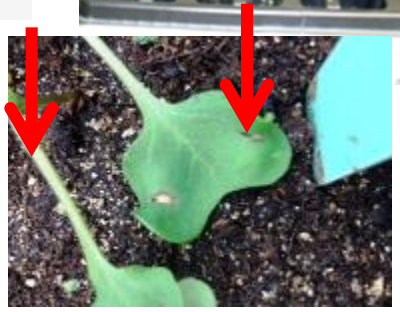
The pots were placed in flats and kept in the growth chamber at 22/16 C (day/night) with a 16 h photoperiod

14 DAP plants inoculated on cotyl. or petiole kept dew chamber for 24 h before back to GC

Isolate	*AvrLm*	Westar	CCC1	CCC2	CCC3
S7	*1,5,6,7(8)*	5.7	1.0	4.0	4.7
P27D	*1,5,6,7(8),10*	6.7	1.0	3.3	4.0
V45-30	*2,7,(10)*	7.9	5.9	6.2	7.0
19.4.24	*3,5,6,8,(10)*	7.3	1.3	1.0	3.3
V23-2-1	*4,5,6,7,8,(10)*	8.1	6.1	7.0	7.0
IBCN	*5,6*	7.4	7.0	7.0	6.6
290CDN	*5,6,7,8,10,Lep 2*	7.3	5.0	5.0	6.0
NZT-4	*5,6,8,(10)*	9.0	5.9	5.7	7.9
PHW1223	*5,6,8,9*	7.4	5.4	7.0	7.0
R2	*5,7,(8),10*	7.4	5.4	7.0	7.0





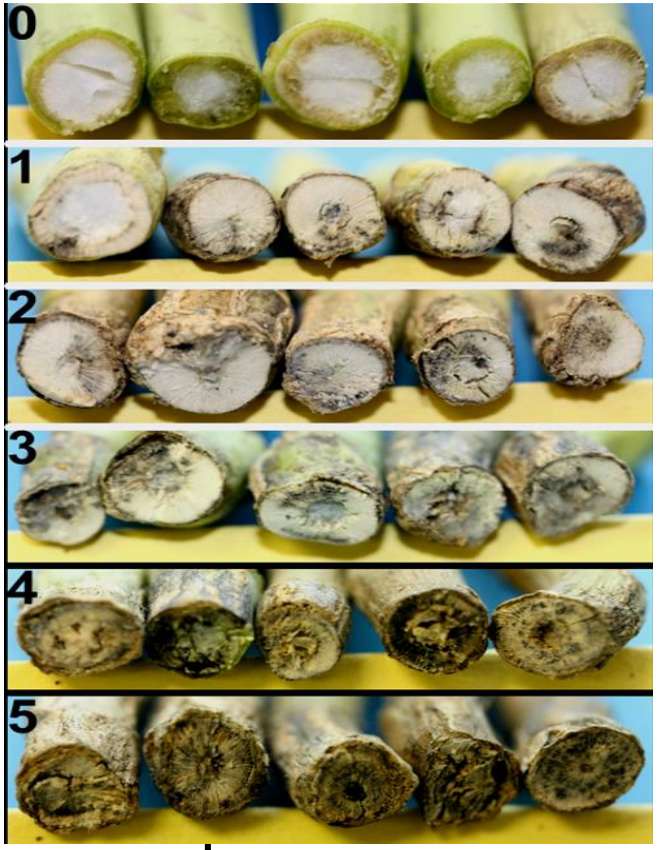


Seven days post inoculation (dpi) the plants were repotted into 5” pots and randomly placed on the greenhouse bench until early maturity

The plants were monitored from 21 to 56 dpi, at seven day intervals plants were counted and recorded, any plant that died was removed

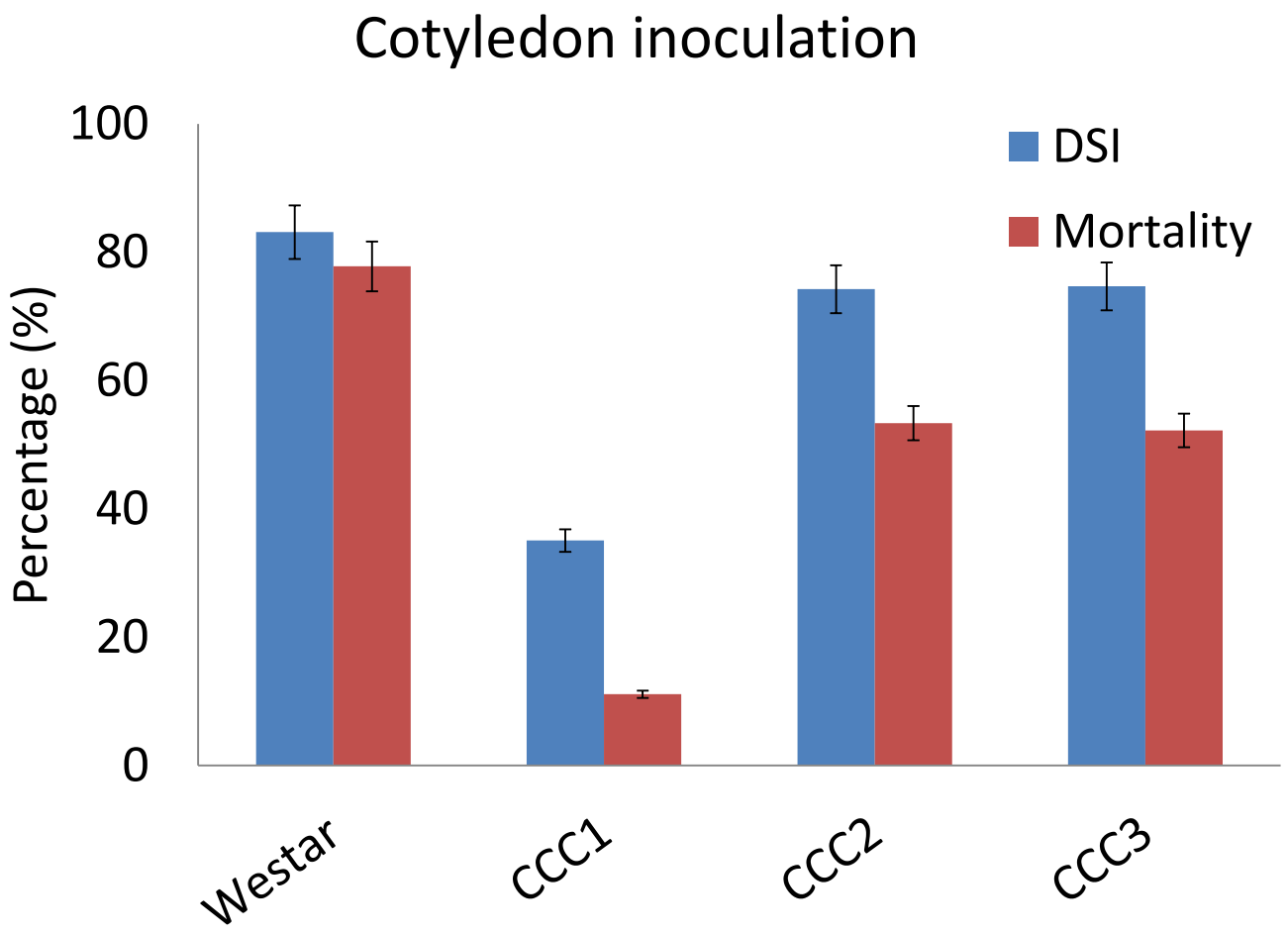
At early maturity, all plants were cut at the base of stem to measure disease severity (DS) using a 0-5 scale.

Each trial was repeated three times



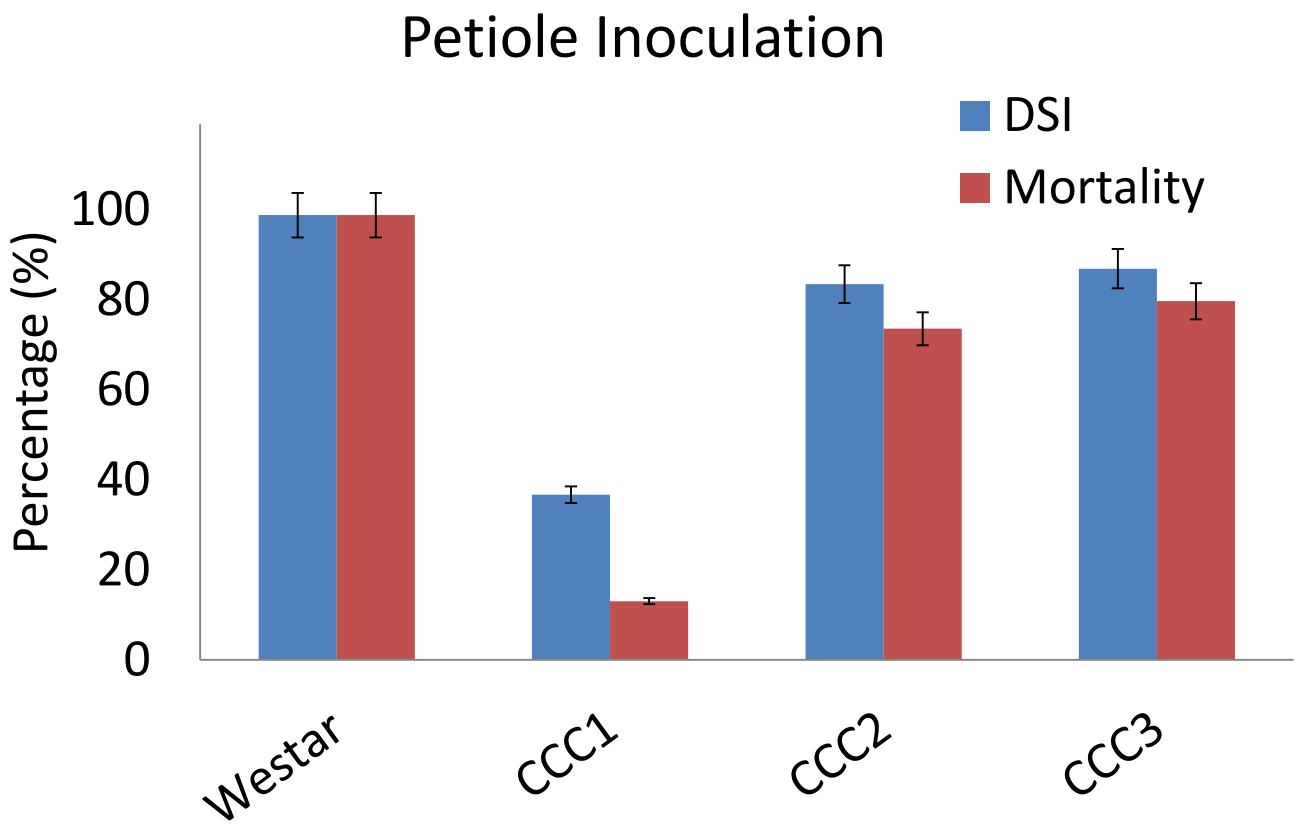
where 0 = no disease and
5 = plant dead

(WCC/RRC, 2009)



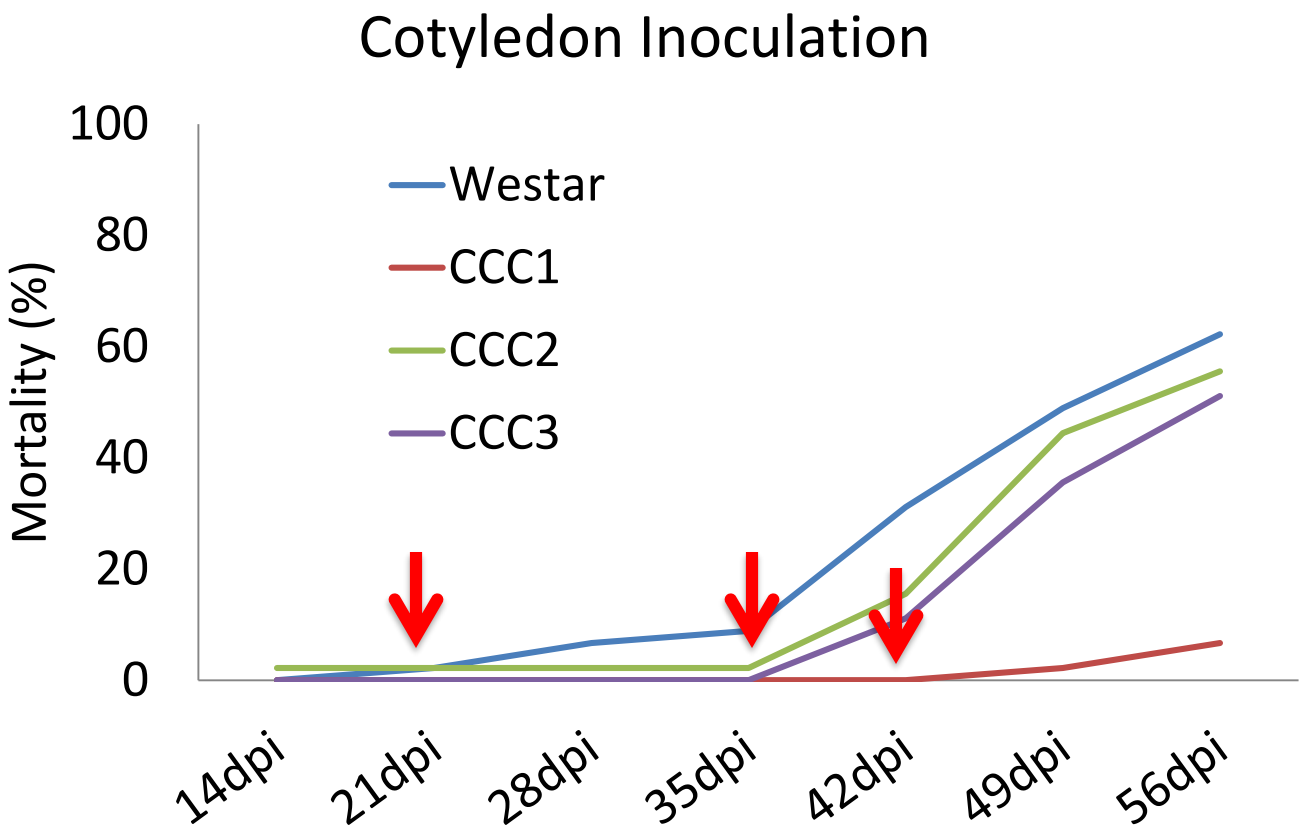
DSI and mortality is higher in Westar and lower in CCCs

It suggests that the development of disease in CCCs stems is slower, compared to Westar

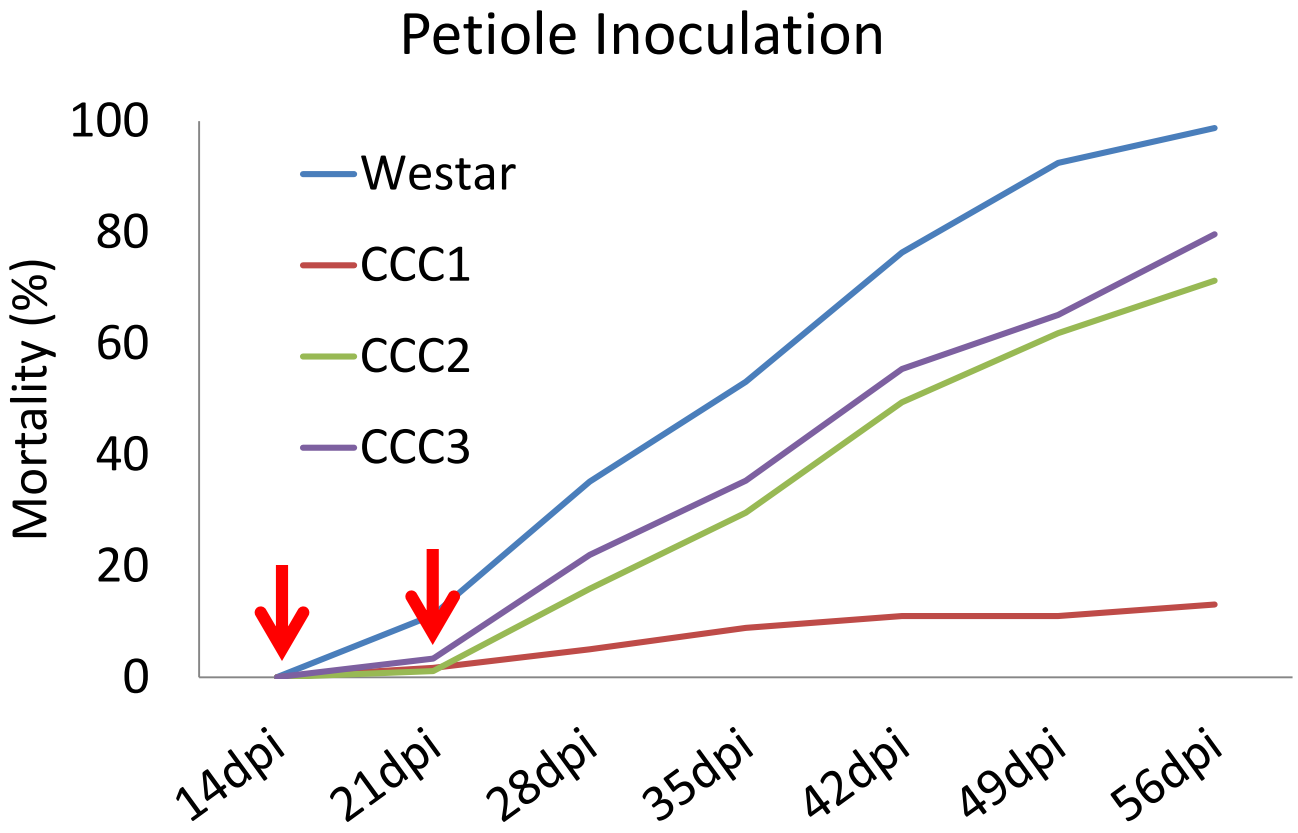


In the petiole inoculation, the pattern was similar to the cotyledon inoculation, but the severity was higher

CCC1 was most resistant; with lowest DSI and mortality

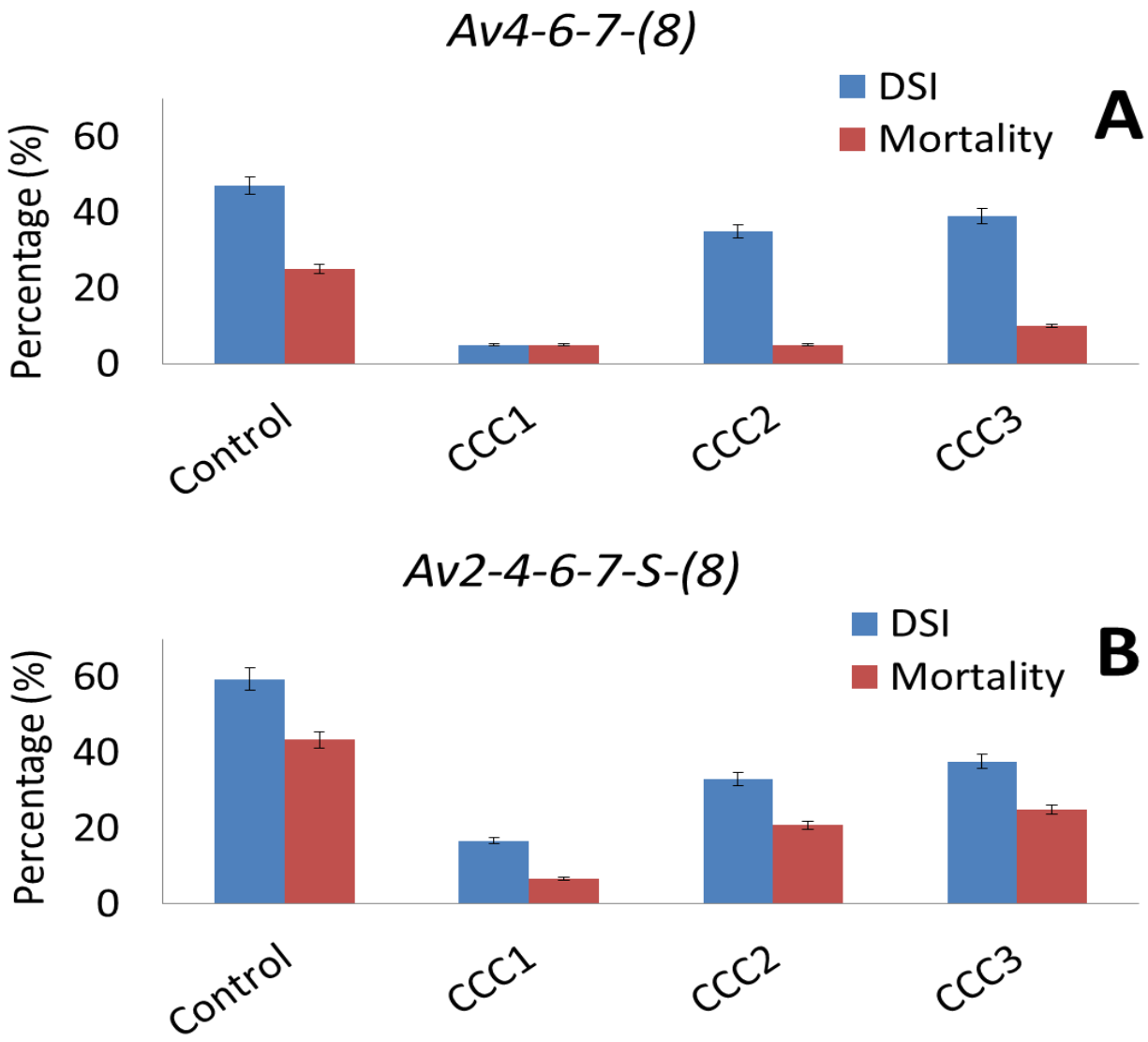


Onset of mortality in Westar occurred at 21 dpi, but in CCC2 and CCC3 it occurred at 35 dpi, and for CCC1 at 42 dpi



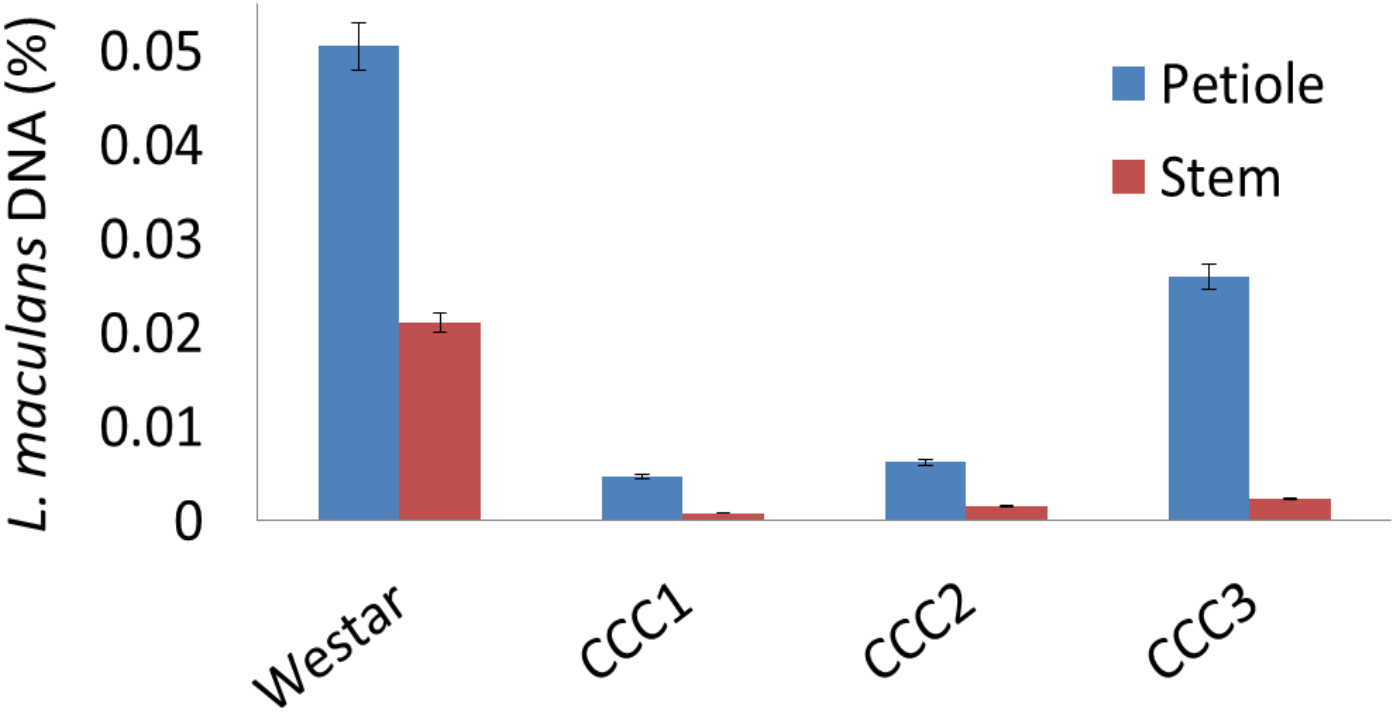
In petiole inoculation the onset of mortality was at 14 dpi for Westar and 21 dpi for the CCCs

Cotyledon inoculation with additional virulent *L. maculans* isolates



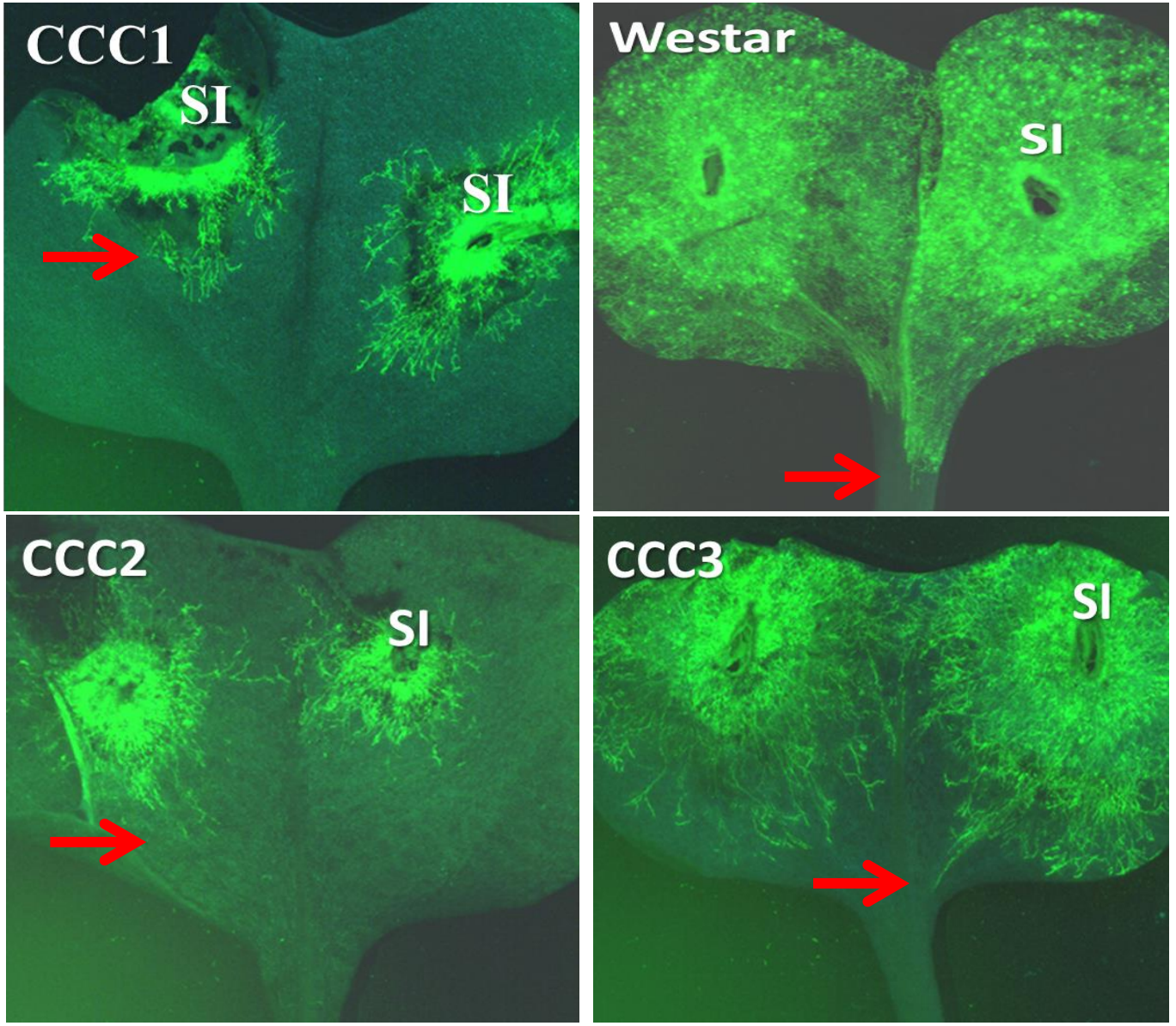
The results suggest that the CCCs limit pathogen movement from the infected cotyledon to the petiole and stem

To assess pathogen movement, ddPCR was adopted to quantify the amount of pathogen DNA in the petioles and stems



Ratio of pathogen DNA in the petiole and stem of CCCs and susceptible control (Westar)

GFP labelled Microscopy



Summary

The CCC showed lower DSI and delayed onset of mortality in both cotyledon and petiole inoculation trials, relative to the susceptible control

L. maculans spread more slowly from infected cotyledons into the petioles/stem of CCCs

The infection development in the stem of CCCs was much slower

Conclusion

Study 1

Based on *Avr* gene profiling, *Av2*, *Av4*, *Av6* and *Av7* are generally common in most fields, regardless of blackleg incidence and severity

This study showed that *Av1*, *Av3*, *Av9* and *AvrLep2* were at very low levels or even absent in most of the fields examined

This indicated that corresponding *Rlm* genes in canola cultivars are not very effective in any of these fields

Conclusion

Study 2

Movement of the pathogen from infected cotyledons to the petiole and stem is slower in the CCCs than in the susceptible control

Blackleg development was also slower in the CCC's stems, which also indicated stem resistance

Take home message

The current study has highlighted the effectiveness of ddPCR in quantifying the spread of *L.* maculans infection in petioles and stems of canola, which appears to correlate to quantitative resistance (*QR)* expression of CCC.

It may be feasible to develop a ddPCR-based protocol for efficient screening of *QR* in commercial breeding lines to continue improving nonspecific blackleg resistance in CCCs

Acknowledgements

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Thank you very much

Any Questions!!